Application No. 10/525,726 Amendment dated October 16, 2007 Reply to Office Action of July 16, 2007

AMENDMENTS TO THE SPECIFICATION

Docket No.: 37998-237386

The Examiner has asked that the sequences listed on pages 27-28 of the specification be labeled appropriately. Accordingly, Applicant hereby submits the following replacement paragraphs which number the sequences in the proper format. Applicant requests that paragraphs [0078], [0079], [0080], and [0081] as filed be replaced with the following paragraphs, which have been marked up to show the changes made. No new matter has been added.

[0078] First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the foap-13 gene: 5'-TCAGGTGAAGAGTGAGGTTGTCA-3' <u>SEQ ID NO. 7</u> and 5'-GGCTGCACTCTTGAGGGAGA-3' <u>SEQ ID NO. 8</u>.

[0079] PCR amplification (95 °C and 1 sec, 56 °C. and 5 sec, and 72 °C. and 5 sec) was performed in a volume of 20 µl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM MgCl₂; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM MgCl₂. Melting curve analysis revealed a single peak at approximately 83 °C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 66 bp for the foap-13 gene was observed in the electropherogram of the sample.

[0080] In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' SEQ ID NO. 9 and 5'-

AGCCGTTGGTGTCTTTGCC-3' <u>SEQ ID NO. 10</u> except for MgCl₂ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the

#900387

Application No. 10/525,726 Amendment dated October 16, 2007 Reply to Office Action of July 16, 2007

expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' <u>SEQ ID NO. 11</u> and 5'-TCTCATCAAGCGTCAGCAGTTC-3' <u>SEQ ID NO. 12</u> (exception: additional 1 mM MgCl₂ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85 °C with no visible primer dimers.

Docket No.: 37998-237386

[0081] Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' SEQ ID NO. 13 and 5'-GGCAAGGGACTTCCTGTAA-3' SEQ ID NO. 14. Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGAACCATG-3' SEQ ID NO. 15 and 5'-GCTAAGCAGTTGGTGGAGCAG-3' SEQ ID NO. 16. Melting curve analysis revealed a single peak at approximately 83 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' SEQ ID NO. 17 and 5'-AGCAGTTGGCTGTTGTACCTCCT-3' SEQ ID NO. 18. Melting curve analysis revealed a single peak at approximately 83 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

#900387